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GENETIC CHARACTERIZATION OF SEVERAL PROMISING ACCESSION OF *Jatropha curcas* L. BASED ON RAPD MARKER

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ABSTRACT

The objective of this research was to obtain genetic relationship among 13 *Jatropha curcas* L. accession plants based on Random Amplified Polymorphic DNA marker. This experiment used 13 accessions of *J. curcas* L. potential to have higher seed productivity, including HS-49, SP-16, SP-38, SP-8, SM-33, SP-34, SM-35, IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, and IP-2P. Polymerase Chain Reaction (PCR) was performed using 10 selected primers of RAPD markers (OPA 2, OPA 9, OPA 13, OPA 15, OPA 18, OPA 19, OPA 20, OPF 8, OPF 10, and OPF 15). PCR product was used to determine genetic distance which implemented Unweighted Pair-Group Method With Arithmetic (UPGMA) procedure and constructed phylogeny trees using Numerical Taxonomy and Multivariate System (NTSYS) software version 1.8. The confidence level of UPGMA was then tested by Boostrap using WinBoot program. Ten primers used in this research were able to be applied in genomic DNA of *J. curcas L.* plant which had resulted about four (OPA 19) to ten band numbers (OPA 9) with the band size around 72-1,078 bp. However, OPA 13 primer was not able to give different band size. Genetic relationship analysis has found two main groups, firstly accession plants consisted of HS-49, SP-16, SP-18, SP-8, SM-33, SM-35, and SP-34 (coefficient 0.8). In this group, SP-38 clustered with SP-8, and SM-33 with SM-35 (coefficient 0.91). In the second group, the accessions consisted of IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, and IP-2P (coefficient 0.78). In this group, accession of IP-1A clustered with IP-1M (coefficient 0.85), IP-1P with IP-2M (coefficient 0.87), and IP-2A with IP-2P (coefficient 0.90). Then, the first and second groups formed genetic relationship with coefficient 0.66.

Key words: Genetic characterization, *Jatropha curcas* L., RAPD, molecular marker, promising accession.

ABSTRAK

Penelitian ini bertujuan untuk mendapatkan informasi keragaman genetik dan hubungan kekerabatan berbagai aksesi jarak pagar terpilih berdasarkan analisis molekuler *Random Amplified Polymorphic DNA* (RAPD). Penelitian menggunakan 13 aksesi *Jatropha curcas* yang memiliki potensi produksi tinggi (HS-49, SP-16, SP-38, SP-8, SM-33, SP-34, SM-35, IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, dan IP-2P). Isolasi DNA genom *J. curcas* dilaksanakan dengan metode Zheng yang dimodifikasi. *Polymerase Chain Reaction* (PCR) dilakukan menggunakan 10 primers RAPD (primer OPA 2, OPA 9, OPA 13, OPA 15, OPA 18, OPA 19, OPA 20, OPF 8, OPF 10, dan OPF 15). Produk PCR yang dihasilkan digunakan untuk menentukan tingkat kekerabatan menggunakan *Un-weighted Pair-Group Method With Arithmetic (*UPGMA) dan diagram filogenetik dengan program *Numerical Taxonomy and Multivariate System* (NTSYS*)* versi 1.8. Kesepuluh primer yang digunakan mampu mengamplifikasi DNA jarak pagar dengan jumlah produk pita antara 4 (primer OPA 19) hingga 10 pita DNA (OPA 9), dengan ukuran pita 72-1.078 bp. Primer OPA 13 tidak dapat memberikan perbedaan pita DNA. Hasil analisis kekerabatan menunjukkan adanya dua kelompok utama. Kelompok pertama terdiri atas aksesi HS-49, SP-16, SP-18, SP-8, SM-33, SM-35, dan SP-34 (koefisien 0,80). Dalam kelompok pertama, SP-38 berkelompok dengan SP-8, dan SM-33 dengan SM-35 (koefisien 0,91). Kelompok kedua terdiri atas aksesi IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, dan IP-2P (koefisien 0,78). Dalam kelompok kedua, IP-1A berkelompok dengan IP-1M (koefisien 0,85), IP-1P dengan IP-2M (koefisien 0,87), dan IP-2A dengan IP-2P (koefisien 0,90). Selanjutnya, kelompok pertama dan kelompok kedua membentuk kekerabatan pada koefisien 0,66.

Kata kunci: Karakterisasi genetik, *Jatropha curcas* L., RAPD, marka molekuler, aksesi harapan.

INTRODUCTION

Supplies of crude oils tend to result in increasing the need for increased utilization of renewable energy sources as an important part of the energy diversification program. Bio-fuels (bio-diesel and bio-ethanol) are the options that can be used as oil substitute energy sources. Utilization of bio-fuels in addition to reducing dependence on oil (energy diversification) have also positive impacts on the environment because bio-fuels are fuels of low pollutant emissions, biodegradable, non-toxic (MORGAN, 2005), and are able to reduce greenhouse gases emissions until it reaches 90 percent (THEIL, S. 2005). *J. curcas* L. is a member of euphorbiacae families. Genus jatropha has 175 species and five of which are in Indonesia : *J. curcas* L., *J. gossypiifolia*, *J. integerrima* Jacq., *J. multifida*, and *J. podagrica* Hook (PUSLITBANG PERKEBUNAN, 2006). Oil content in *J. curcas* is high enough so that it can be used to substitute diesel oil. The potential use of bio-diesel as alternative fuel or diesel oil or a mixture of automotive diesel oil in the transportation sector will continue to rise and is predicted until the year of 2025 increasing to reach 57 percent of the total use of diesel oil in the sector. In 2025, bio-diesel demand in Indonesia is estimated to reach a total of more than 281 PJ, which is equivalent to seven million tons or 6 million kiloliters of bio-diesel (BPPT, 2006).

Superior variety is needed, but until now there is not sufficient high yielding varieties (SUDARMO *et al.*, 2006) and in Indonesia there is no variety or clone produced jatropha yet (HARIYADI, 2005). Improvement of varieties can be implemented if there are adequate sources of germplasm. In management of germplasm, it is necessary to study the characters of each accession through activities both in the phenotypic characterization and molecular, therefore molecular characterization is needed in breeding programs. Local germplasm diversity will be used for variety improvement if information on patterns of genetic diversity and phylogenetic relationships among *J. curcas* varieties, both phenotypic and molecular, is available. Molecular genetic approaches using DNA characterization successfully establish a molecular marker capable of detecting genes and particular traits, evaluation of genetic diversity, kinship, and the existence of evolution at the genetic level (HOON-LIM *et al.*, 1999).

The ability to distinguish individual genotype within the species and some genotypes accurately is needed in plant breeding programs. Morphological characteristics and the phenotype have been widely used, but quantitative traits controlled by many genes are generally strongly influenced by the environment so that differences among closely related species are often difficult to observe. Most of the characters are difficult to analyze because they do not have simple genetic control systems. Use of molecular markers can help to overcome these problems. Molecular markers based on DNA banding patterns have been widely used to construct phylogenetic some individuals within the species and kinship among species. Kinship can be used as reference in cross breeding to obtain a high diversity of the crosses. The use of DNA markers can assist in c arrying the election of elders crossing having high genetic differences (CORREA *et al.*, 1999).

RAPD analysis using primers of ten bases is often used for the study of kinship and identification of varieties (CIMMYT, 1998), genetic mapping, DNA structure analysis of an individual organism and fingerprinting (MAFTUCHAH, 2001). LIU and FURNIER (1993) reported that the use of RAPD showed a higher diversity than alozim and RFLP, thus strongly supports the effort to analyze genetic diversity in crops background genome is not yet known. RAPD technique has been used to improve the efficiency of selection early on annual crops (GRATTAPAGLIA *et al.*, 1992). RAPD molecular analysis was also used to develop fingerprints and genetic relationships, to develop kinship, some individuals within and among species, and has been used on various crops including *J. curcas* (MAFTUCHAH, 2006). At the beginning of the research activities on the jatropha plant molecular analysis based on RAPD markers, it has been generated randomly amplified polymorphic DNA fingerprint pattern for the local jatropha plant from Karangtengah, NTB, and Lamongan. Results of selection of the 40 RAPD primers showed that 14 primers used were suitable for *J. curcas* DNA amplification (MAFTUCHAH and ZAINUDIN, 2006). In addition, it has obtained the appropriate number of primers in DNA amplification processes of Lamongan, Karangtengah, and NTB accessions with a high level of polymorphism (MAFTUCHAH and ZAINUDIN, 2006). Fourteen RAPD primers were used to produce the total number of 75, 91, and 60 DNA bands on Karangtengah, Lamongan, and NTB accessions, respectively, and the size of DNA bands were between 200 to 642 bp (MAFTUCHAH and ZAINUDIN, 2007-a). Primer OPA provides the number of DNA bands for more than OPF, but use of some types of primer OPA is sometimes not able to provide different patterns of DNA bands between *J. curcas* of Karangtengah and Lamongan accessions (MAFTUCHAH and ZAINUDIN, 2007b).

From the farmer research, it was obtained that seven *J. curcas* accessions had high productivity, namely HS-49 (1,097.50 kg/ha), SP-16 (977.50 kg/ha), SP-38 (912.50 kg/ha), SP-8 (656.07 kg/ha), SM- 33 (622.50 kg/ha), SP-34 (578.33 kg/ha), and SM-35 (500 kg/ha) (SUDARMO, *et al.*, 2006). From the results of mass selection, they produced some of IP1 accessions with the productivity potential of 4-5 t/ha and IP2 accessions with production potential of 7- 8 t/ha in the fifth year (HASNAM, 2006). The area for plant development of *J. curcas* is an effort to supply bio-diesel in Indonesia. It is projected around 2.4 million ha up to year 2025, but land take precedence jatropha cultivation in upland and non-productive (PUSLITBANG PERKEBUNAN, 2006).

The purpose of this study was to find out the information on 13 accessions of kinship promising jatropha based on molecular analysis on Random Amplified Polymorphic DNA (RAPD).

MATERIALS AND METHODS

Accession of *J. curcas* used in this study originated from plants of germplasm collection located at Asembagus - Situbondo. Molecular analysis of *J. curcas* was conducted at the Laboratory of Molecular Plant Biotechnology Development Center - University Muhammadiyah of Malang, in May – September 2008. Research was carried out by using 13 accessions of local *J. curcas* which have high productivity, namely HS-49, SP-16, SP-38, SP-8, SM-33, SP-34, and SM-35 (SUDARMO *et al.*, 2006) and the IP-1A, IP-1M, IP-1P, IP1, IP2-A,-M and IP2 IP-2P (HASNAM, 2006). RAPD molecular analysis was carried out by using the Random Amplified Polymorphic primer 10 DNA, each measuring ten bases (primer OPA 2, OPA 9, OPA 13, OPA 15, OPA 18, OPA 19, OPA 20, OPF 8, OPF 10, and OPF 15) (MAFTUCHAH and ZAINUDIN, 2006).

Plant Genomic DNA Isolation. Plant genomic DNA was isolated from young leaves of selected *J. curcas* accessions.

The procedures for genomic DNA isolation and purification from plant leaves of *J. curcas* were conducted according to the modified of Zheng method (ZAINUDIN and MAFTUCHAH, 2006). After the isolated DNA was purified, the concentration was calculated using spectrophotometer. Subsequently the DNA was dissolved in TE solution and stored in 40°C temperatures. Jatropha plant genomic DNA isolated subsequently was detected through the process of DNA electrophoresis in 1% agarose gel with a voltage of 100 V and running time of 1 hour. Gel was then performed with Polaroid photography. In the next activity, genomic DNA was ready to be used as a template in the reaction of Polymerase Chain Reaction (PCR).

PCR Reaction. In PCR reactions, genomic DNA jatropha plant was used as a template. The ten primers used in this PCR reaction each measured ten bases (MAFTUCHAH and ZAINUDIN, 2006). The volume of PCR reaction employed was about 25 mixture solutions consisting of taq DNA polymerase and 10X taq polymerase buffer (100 mM Tris-Cl, pH 3.8, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin); dNTPs's mix (dGTP, dATP, dTTP and dCTP) (Pharmacia); dH_2O and 30 ηg template DNA with PCR conditions according to preliminary research results (MAFTUCHAH and ZAINUDIN, 2006). The amplified genomic DNA of plant spacing on subsequent PCR process was detected by 2% agarose gel electrophoresis.

Alleles Detection and Phylogenic Analysis. Detection of RAPD alleles and phylogenic analysis of DNA-RAPD fingerprints was conducted based on the number, frequency, and distribution of DNA alleles based on RAPD markers. Detected fragments from different DNA banding pattern were resulted from the agarose gel electrophoresis of PCR products. DNA bands positioning was done manually (LEUNG *et al.*, 1993). Implementation stages were as follows: 1) All DNA bands with the same migration rate were assumed as a locus of homologus, 2) Each of the DNA band was marked (can use colored ink), each represented one sign of a particular DNA band position, which was done by attaching a transparent plastic on the gel images, 3) If DNA path separate from one another, find the tools to help determine the position of the DNA bands, 4) Data were the DNA profiles of the observed allele with existing regulations and the DNA bands based on the size of the PCR product in a similar position from a few individuals *J. curcas* were compared bands that appeared on the gel were assumed as a RAPD allele. The diversity of RAPD alleles was determined from the difference in gel migration of alleles from each individual sample. Based on the presence or absence of RAPD bands, band profiles were translated into binary data. For the preparation of the binary, data matrix were reduced to a matrix of genetic similarity (NEI and LI, 1979). Clustering and dendogram analysis was performed using the method of Un-weighted Pair-Group Method with Arithmetic (UPGMA) through the program of Numerical Taxonomy and Multivariate System (NTSYS) version 1.8. Degree of accuracy data was analyzed by UPGMA analysis with bootstrap analysis using WinBoot program. It was expected to get specific information for *J. curcas*.

Kinship varieties tested were determined based on the score of the genetic distance between cultivars i and j (dij) based on the presence or absence of DNA bands. Score 1 if there was a ribbon of DNA and score 0 if no DNA bands. Equation of Nei and Li's similarity coefficient (NEI and LI, 1979) :

$$Dij = 1 - \frac{2a}{2a + b + c}$$

where Dij = dissimilarity coefficient, Sij = similarity coefficient, = number of pairs of 1.1; = number of pairs of 1.0; = number of pairs of 0.1.

RESULTS AND DISCUSSION

Genomic DNA isolated from young leaves of 13 accessions of *Jatropha curcas* was used in this study. The isolated DNA was ready to be used as a template in the Polymerase Chain Reaction. In PCR, genomic DNA of *J. curcas* plants was used as a template and in this PCR, it used ten primers which each measured ten bases (MAFTUCHAH and ZAINUDIN, 2006).

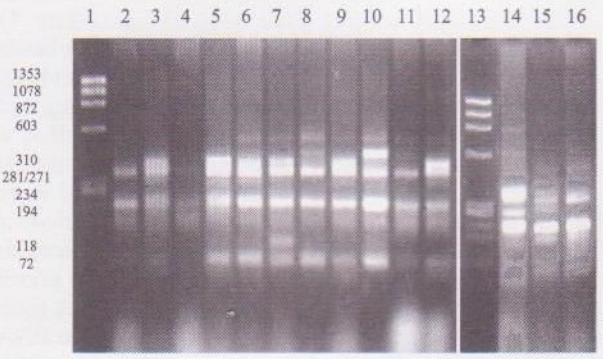

Figure 1. DNA fingerprints of jatropha (*Jatropha curcas* L)
Gambar 1. Fingerprint dari DNA jarak pagar (Jatropha curcas L)

Note *Keterangan* : Marker/*penanda* 100 bp (line/*lajur* 1), HS 49 + OPA2 (row/*baris* 2), SP 16 + OPA2 (row/*baris* 3), SP 38 + OPA2 (row/*baris* 4), SP 8 + OPA2 (line/*lajur* 5), SM 33 + OPA2 (line/*lajur* 6), SP 34 + OPA2 (line/*lajur* 7), SM 35 + OPA2 (line/*lajur* 8), IP-1A + OPA2 (line/*lajur* 9), IP-1M + OPA2 (line/*lajur* 10), IP-1P + OPA2 (line/*lajur* 11), IP-2A + OPA2 (line/*lajur* 12), marker 100 bp (line/*lajur* 13), IP-2M + OPA2 (line/*lajur* 14), IP-2P + OPA2 (line/*lajur* 15), SP 38 + OPA2 (line/*lajur* 16)

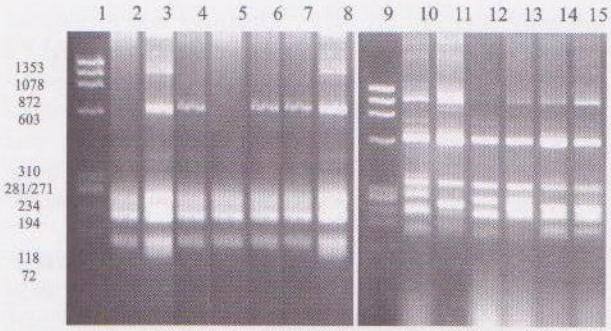

Figure 2. DNA fingerprints jatropha (*Jatropha curcas* L)
Gambar 2. Fingerprint dari DNA jarak pagar (Jatropha curcas L)

Note *Keterangan* : Marker/*penanda* 100 bp (line/*lajur* 1), HS 49 + OPA9 (row/*baris* 2), SP 16 + OPA9 (row/ *baris* 3), SP 38 + OPA9 (row/*baris* 4), SP 8 + OPA9 (line/ *lajur* 5), SM 33 + OPA9 (line/*lajur* 6), SP 34 + OPA9 (line/*lajur* 7), SM 35 + OPA9 (line/*lajur* 8), marker 100 bp (line/*lajur* 9), IP-1A + OPA9 (line/ *lajur* 10), IP-1M + OPA9 (line/*lajur* 11), IP-1P + OPA9 (line/ *lajur* 12), IP-2A + OPA9 (line/ *lajur* 13), IP-2M + OPA9 (line/*lajur* 14), IP-2P + OPA9 (line/ *lajur* 15)

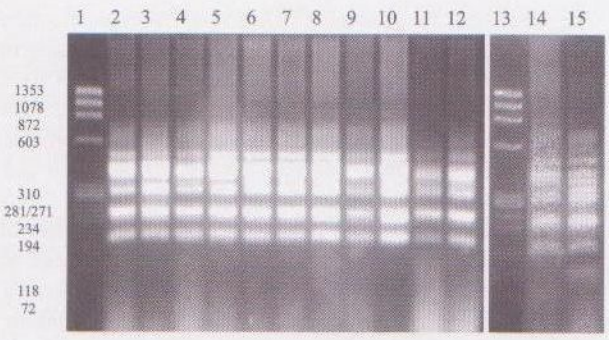

Figure 3. DNA fingerprints jatropha (*Jatropha curcas* L)
Gambar 3. Fingerprint dari DNA jarak pagar (Jatropha curcas L)

Note *Keterangan* : Marker/*penanda* 100 bp (line/*lajur* 1), HS 49 + OPA13 (row/*baris* 2), SP 16 + OPA13 (row/ *baris* 3), SP 38 + OPA13 (row/ *baris* 4), SP 8 + OPA13 (line/*lajur* 5), SM 33 + OPA13 (line/*lajur* 6), SP 34 + OPA13 (line/*lajur* 7), SM 35 + OPA13 (line/*lajur* 8), IP-1A + OPA13 (line/ *lajur* 9), IP-1M + OPA13 (line/*lajur* 10), IP-1P + OPA13 (line/ *lajur* 11), IP-2A + OPA13 (line/*lajur* 12), marker 100 bp (line/*lajur* 13), IP-2M + OPA13 (line/*lajur* 14), IP-2P + OPA13 (line/*lajur* 15)

Results showed that all ten primers, used to amplify genomic DNA *J. curcas* with the number and size of DNA bands, were very diverse. Of the ten primers used, it had been generated 65 DNA bands from the thirteen *J. curcas* accessions tested. Overall banding pattern that was formed subsequently was used in preparing the phylogenic analysis among the thirteen accessions.

Figure 1 shows the DNA-RAPD banding pattern of *J. curcas* plant using primer OPA 2, while Figure 2 is a DNA-RAPD banding pattern of *J. curcas* plant using primer OPA 9. Use of primer OPA 2 produced nine DNA bands with sizes ranging from 194-603 bp (Figure 1). While the primary use of OPA 9 produced 10 DNA bands of 194-1,078 bp sizes (Figure 2). LIU and FURNIER (1993) stated that the use of RAPD showed a higher diversity than alozim and RFLP did, thus strongly supports the effort to analyze genetic diversity in crops background genome has not yet been known. Using primer OPA 13 proved to be unable to provide DNA differences among the thirteen *J. curcas* accessions tested. All of the accessions produced six DNA bands with a uniform size (Figure 3), and the primer OPA 15 was able to produce seven DNA bands with sizes between 194-872 bp. Primer OPA 18 produced seven DNA bands with sizes between 118-603 bp, while primer OPA 19 produced only seven DNA bands.

Results of DNA amplification *J. curcas* using 10 random primers did not always produce the band with same intensity. Differences in intensity of each band can not be used to estimate the amount of coffee base pairs at each RAPD band. The intensity of DNA bands amplified in each primer was strongly influenced by the purity and concentration of template DNA and the distribution of primary attachment site on the template DNA (GRATTAPAGLIA *et al.*, 1992).

The RAPD molecular analysis techniques have been used to develop fingerprints and genetic relationships, develop kinship, some individuals within and among species, and have been used in a variety of plants including *J.curcas* plants (GRATTAPAGLIA *et al.*, 1992; MAFTUCHAH, 2006). DNA fingerprint analyses based on the results of PCR using primer 10 implemented based on the number, frequency, and distribution of DNA alleles are formed. Detected fragments of DNA banding pattern were different from the results of agarose gel electrophoresis of PCR products and DNA bands positioning is done manually (LEUNG *et al.*, 1993).

Figure 4 shows the dendrogram of 13 *J. curcas* accessions cluster analysis using UPGMA based on RAPD banding pattern using 10 primers. From the results of phylo genic analysis showed two main groups. The first group consisted of accessions of HS-49, SP-16, SP-18, SP-8, SM-33, SM-35, and SP-34 (with a coefficient of 0.8). In the first group, 38 accessions clustered with accessions SP-8, and accession clustered with SM-33 and SM-35 (with a coefficient of 0.91). The second group consists of accessions IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, and IP-2P (coefficient 0.78). In this second group, IP-1M accessions clustered with IP-1A, IP-1P grouped with IP-2M, and IP-2A grouped with IP-2P. Furthermore, the first group (HS-49, SP-16, SP-18, SP-8, SM-33, SM-35, and SP-34) and the second group (IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, and IP-2P) formed the kinship with coefficient of 0.66.

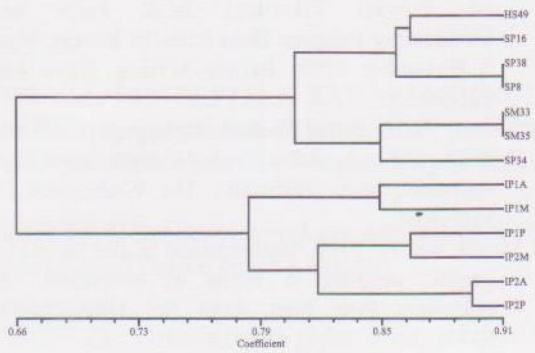

Figure 4. Dendrogram of 13 accessions of jatropha plant cluster analysis using UPGMA based on RAPD banding pattern using 10 primer

Gambar 4. Dendrogram analisis cluster dari 13 aksesi tanaman jarak pagar menggunakan UPGMA berdasarkan pola pita RAPD dari 10 primer

The development of PCR techniques occured quickly after the discovery of the genome double folding technique at several different loci using primers arbitrari, known as RAPD. In plant breeding programs, identification is required both morphological and molecular characters to test the genotypic diversity of clones which are selected for parental crosses. RAPD technique has better resolution compared to RFLP analysis in terms of kinship between the genotypes and can produce an unlimited number of characters that are very helpful in the analysis of genetic variability of unknown background genome (LIU and FURNIER, 1993). RAPD analysis requires only small amounts of DNA making it very suitable for the species of woody plants (ROWLAND and LEVI, 1994). The number of primers required depends on the purpose or type of information desired. The increasing numbers of primers used the lower the coefficient of variation obtained with the use of 10 primary and experimental errors can be reduced to near zero values (PENNER, 1996). RAPD technique using random or specific primers has proven to be used as molecular markers for important agronomic characters. The RAPD molecular markers have been used to construct phylogenetic some individuals within the species and kinship among species. In addition, this kinship pattern can be used as reference in breeding crosses to obtain a high variability of the results of a hybridization process.

CONCLUSION

Ten primers used in this research could be applied in genomic DNA of *J.curcas L.* plant which has resulted about four (OPA 19) to ten (OPA 9) bands with the sizes around 72-1,078 bp and OPA 13 primer could not give different band of the 13 accession plants of *J.curcas* L. Genetic relationship analysis has found two main groups. First group of accession plants consisted of HS-49, SP-16, SP-18, SP-8, SM-33, SM-35, and SP-34 (coefficient 0.8). In this group, SP-38 clustered with SP-8 and SM-33 with SM-35 (coefficient 0.91). In the second group, the accessions consisted of IP-1A, IP-1M, IP-1P, IP-2A, IP-2M, and IP-2P (coefficient 0.78). In this group, accession of IP1A clustered with IP-1M (coefficient 0.85), IP-1P with IP-2M (coefficient 0.87), and IP-2A with IP-2P (coefficient 0.90). Then, the first and second groups formed genetic relationship with coefficient 0.66.

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